

The Relevance of Immunogenicity in Preclinical Development

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Abstract

This communication discusses the relevance of immunogenicity in preclinical trials and its importance in the development of biopharmaceuticals. The preclinical and non clinical development of Biopharmaceuticals is reviewed and discussed, with a focus on the specific regulatory requirements for the preclinical and clinical safety assessment of large molecules. Immunogenicity data obtained for Interferon and Rituximab during preclinical safety assessment studies are presented in this communication. Interferon and Rituximab are biopharmaceuticals that have been demonstrated to elicit immunogenic reactions in both preclinical animal models and clinical trials. The production of Anti-Drug Antibodies (ADAs) has several consequences on the systemic exposure and on the toxicity/pharmacodynamic profile of a biopharmaceutical assessed in the preclinical stage. The impact must be carefully assessed especially when using the preclinical data to establish the Maximum Recommended Safe Starting Dose (MRSD) of the first clinical trials.

Keywords: Immunogenicity; Non-human primates; Rituximab; Interferon; Preclinical; Safety; Biopharmaceuticals

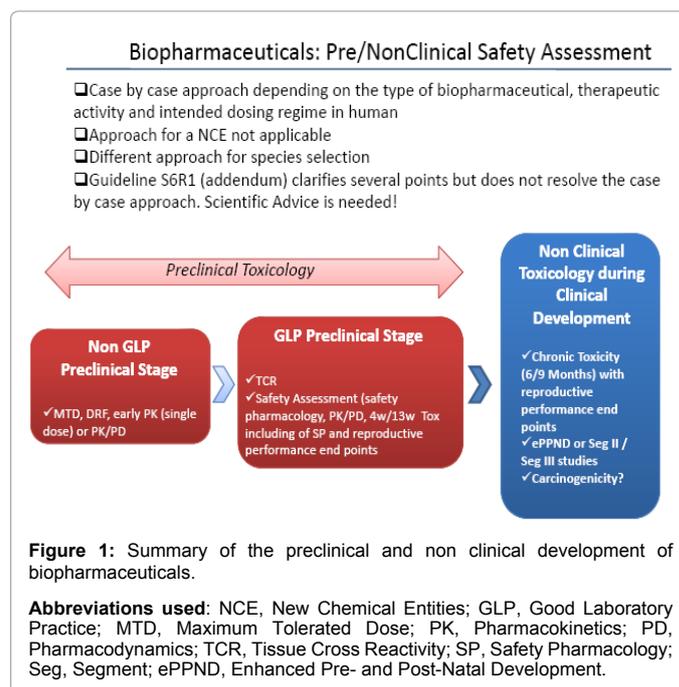
Introduction

The preclinical development of biopharmaceuticals or biotechnology-derived pharmaceuticals such as monoclonal antibodies, recombinant proteins, growth factors, etc. does not follow the “standard” rule as opposed to small molecules or new chemical entities. In addition, the biopharmaceuticals, being in essence large molecules, are susceptible to be targeted by the immune system eliciting an immunogenic reaction which can lead to adverse and even lethal and irreversible effects. This communication will discuss the cause of the onset of an immunogenic reaction and its clinical relevance when detected during the preclinical toxicity testing.

Preclinical Development of Biopharmaceuticals

In general, the toxicity profiling of biological molecules or biopharmaceuticals during the regulatory preclinical development is divided in two blocks, one consisting in a series of preliminary toxicity studies like the Dose Range Finder (DRF), the Maximum Tolerated Dose (MTD) and an early assessment of the pharmacokinetic behavior associated or not with a pharmacodynamic assessment. Most of those studies performed at this stage of the toxicity profiling are not required to be performed under Good Laboratory Practice. The second block of toxicology studies would consist of subacute and/or subchronic toxicity studies including safety pharmacology, pharmacodynamic and reproduction performance end points, stand-alone safety pharmacology studies (telemetry studies), pharmacokinetic studies. Non-clinical toxicity studies must be performed when the biological drug candidate is progressing through the clinical development (from Phase I to Phase III) i.e., chronic toxicity studies and reproduction toxicology studies (enhanced pre- and postnatal development studies in non human primates or the classical segment II and III reproduction toxicology studies). In general, the duration of chronic toxicity studies for a biopharmaceutical does not exceed 6 months of duration and carcinogenicity testing is not required. Tissue cross-reactivity might be performed if epitope recognition and interaction is part of the mechanism of action of the biological drug candidate such as a monoclonal antibody, this latter test being used to complement the rationale for the selection of the species used for the preclinical and non clinical assessment (Figure 1) [1].

The rationale for selecting the appropriate preclinical species for the safety assessment before entering the clinical phase differs between a small molecule and a biological entity. While the rationale for species selection for a new chemical entity is based on metabolic stability and profiling, species selection for the preclinical assessment of a biological molecule is based on pharmacological activity. It makes



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no sense to characterize the toxicity profile of a biological drug in an animal model that is not pharmacologically responsive. In addition, the biopharmaceuticals are not metabolized but catabolized rendering useless or not relevant any “classical” *in vitro* metabolism evaluation and *in vivo* ADME studies [1].

It might be possible that only one species is pharmacologically responsive to the new biological drug candidate e.g. expression of the antigen, appropriate population of B- and T-cells, etc..., thereby justifying the preclinical and non clinical safety assessment in only one animal species being the non human primate in most of the cases.

The standard *in vitro* assessment of cardiotoxicity and genotoxicity are not considered appropriate and meaningful for the biopharmaceuticals [1].

As the biopharmaceuticals are usually administered by the intravenous, intramuscular or subcutaneous route, special attention must be paid to the injection site. Local tolerance must therefore be evaluated and is in general an important part of the toxicology studies, the injection site being also included in the list of the tissues to be assessed histopathologically.

Last but not least, a biological molecule administered parenterally to an animal can be recognized as foreign and will be targeted by the host immune system eventually triggering the so-called immunogenic reaction. This reaction is rather unexpected and unwanted in clinical trials because most of the tested biopharmaceuticals are fully humanized. An immunogenic reaction can take the form of several acute or delayed reactions that can lead to adverse and fatal effects. It is therefore highly recommended to predict and detect as early as possible during the drug development process any signs of immunogenicity.

Immunogenicity: Definition, Causing Factors and Impact on Preclinical Safety Assessment

Immunogenicity is defined as the ability of an antigen to induce an immune response, the antigen being identified as the immunogen. In general, the T-cell response drives the immunogenic reaction which can be transient without clinical significance or lead to adverse effects and maybe life threatening. The hallmarks of an immunogenic response are the production of anti-drug antibodies (ADAs) and/or the triggering of the production of proinflammatory cytokines. After the parenteral administration of a biological molecule, systemic exposure occurs and the molecule might be up taken and processed by antigen-presenting cells (APC). Foreign antigens are presented to CD4+ cells and the interaction MHCII/TCR leads to the activation and proliferation of the T-cells involving also a complex network of cytokines. The activated T-Cells in turn interact with B-Cells triggering their activation and differentiation into plasma cells that produce the antibodies specific to the antigen.

The anti-drug antibodies produced during an immunogenic reaction are IgGs displaying different properties:

- Clearing antibodies: their binding to the tested biological molecule increases its clearance rate and hence shortens its terminal elimination half-life.
- Sustaining antibodies: effect opposed to the effects of the clearing antibodies.
- Neutralizing antibodies (nAbs): their binding neutralizes the pharmacological activity of the tested biological molecule.

- Cross reaction with endogenous proteins leading to auto-immune reactions.
- Indirect implication in the onset of anaphylactic reactions.

Several factors can contribute to the onset of an immunogenic reaction in clinical trials:

- Genotype, age of the patient
- Therapeutic protein sequences
- Uptake by immune cells
- Modification in formulation/manufacturing process eg glycosylation, PEGylation, chemical modification
- Other factors such as pre- and co-medications, route of administration, formulation, dose and frequency of dosing.

The substitution of amino acids compared to the native sequence or the use of “linkers” used as junction in fusion proteins can create novel epitopes prone to be recognized as foreign by the immune system. The PEGylation of a biological molecule can lead to the reduction or neutralization of the activity due to the production of neutralizing antibodies against the polyethylene glycol (PEG) moiety. The absence or altered pattern of glycosylation can expose B- or T-cell epitopes or make the protein foreign to the immune system. The frequency of dosing determines the type of antibodies produced: low affinity IgMs are produced after single dosing (or after the first administration of a repeated dosing regimen) while high affinity IgGs are produced after multiple dosing. An immunogenic reaction can be triggered by repeated injection that can break the immune tolerance to endogenous proteins. The subcutaneous administration is more prone to elicit an immunogenic reaction due to the presence of Langerhans cells in the epidermal tissue. Uncontrolled impurities produced during the manufacturing process can also be the responsible of causing immunogenic reactions.

Human proteins are expected to exhibit minimal or no antigenicity in humans. However, the reverse in animals can happen and is expected. Therefore the production of ADAs in preclinical toxicity studies must be thoroughly assessed and characterized given its impact on the pharmacokinetic behavior and pharmacological effect. The preclinical safety assessment of a tested biological molecule must take into consideration the following aspects in order to draw appropriate conclusions on the preclinical safety profile [2-4]:

- Determination of ADAs in correlation with the systemic exposure (toxicokinetics).
- Isotyping of those ADAs and determination of their neutralizing potential by cell-based assays.
- Careful interpretation of the toxicokinetic profile and pharmacodynamic effect in case of immune mediated clearance or immune mediated sustained exposure.
- Inclusion of late blood collection time points for the determination of ADAs e.g. up to 192h or longer.
- Inclusion of a recovery period of at least 8 weeks (longer than the usual recovery period included in the toxicity studies performed for small molecules) due to the long terminal elimination half-life of the antibodies.
- Careful assessment of the NO(A)EL established for the tested large molecule.

Even if the sequences between the tested large molecule and the host homolog are closely related or even conserved as it could be in the non human primate, the translation of the immunogenic reaction observed in preclinical animal models to the clinical environment is not clear but is not deprived of significance. Immunogenicity also represents a challenge in the development of biosimilars and similarity of the safety profiles between the reference and test item is key before entering the preclinical phase [5].

Interferon and Rituximab

IFN α -2B is a type I interferon consisting of 165 amino acid residues with arginine in position 23 and produced by recombinant DNA technology and resembles the interferon secreted by leukocytes. Interferon plays an important role in the innate immune response and in the establishment of the adaptive response and resistance to a virus infection. IFN α -2B and its pegylated form (PEG-IFN α -2B) display a similar pharmacological activity and are indicated to treat patients with chronic Hepatitis C viral infection or as antineoplastic agents. The molecular mechanism of action passes through the activation of the JAK/STAT pathway for virus infection resistance and activation of caspases for apoptosis induction in malignant cells. 7 to 25% of patients treated subcutaneously with IFN α -2B produce ADAs [6]. Adverse effects reported after the treatment with IFN α -2B or PEG-IFN α -2B are flu-like symptoms occurring 1 to 3 hours after the treatment and caused by proinflammatory cytokines, induction of Systemic Lupus Erythematosus has also been reported.

The clinical advantage of PEG-IFN α -2B results from the fact that it is very slowly cleared after administration, resulting in a longer terminal elimination half-life ($t_{1/2}$) and a higher systemic exposure (both AUC and C_{max}) in both preclinical and clinical studies conferring an increased efficacy compared to IFN α -2B. The pegylation of IFN α -2B also renders the molecule less immunogenic; Pegasys[®] (pegylated Interferon α) is less immunogenic than Roferon[®] (native Interferon α) [6-8]. The toxicokinetic profile of PEG-IFN α -2B obtained in a GLP preclinical toxicology study involving the repeated administration of the biopharmaceutical for 13 weeks by the subcutaneous route in *Macaca fascicularis* (cynomolgus monkey) was characterized as following [9]:

- $t_{1/2}$: 100-180h; 20 to 30 fold longer than the $t_{1/2}$ reported for IFN α -2B.
- Neutralizing antibodies generated already in week 2 of treatment in half of the treated animals (Table 1).
- Toxicokinetic profile showing poor or no systemic exposure to PEG-IFN α -2B from Week 3 of treatment onwards (Figure 2).

Because of the immunogenic reaction affecting the systemic exposure and hence the toxicity profile, the NO(A)EL defined during the preclinical stage for PEG-IFN α -2B must be carefully interpreted and assessed given its importance in defining the Maximum Recommended Starting Dose(MRSD) in Phase I. In addition, the immunogenicity of PEG-IFN α -2B in non human primates suggests a potential immunogenicity in humans that must be followed during the clinical trials.

Another example of preclinical-clinical correlation for immunogenicity is provided by Rituximab. Rituximab is a genetically engineered chimeric murine/human monoclonal antibody directed against the CD20 antigen found on the surface of normal and malignant

B lymphocytes. It is an IgG1 κ immunoglobulin containing murine light- and heavy-chain variable region sequences and human constant region sequences indicated for the treatment of patients with non-Hodgkin's lymphoma (NHL) and chronic leukemia (CLL). The action of rituximab is mediated by effector mechanisms i.e. Complement-Dependent Cytotoxicity (CDC) and Antibody-Dependent Cell-mediated Cytotoxicity (ADCC) but also by apoptosis [10].

The repeated administration of Rituximab to *Macaca fascicularis* (cynomolgus monkey) once weekly for 8 weeks, performed in a GLP preclinical toxicity study, elicited the production of ADAs which majority was neutralizing from week 3 onwards and until the end of the recovery period in half of the treated animals (Table 2) [11]. The systemic exposure to the monoclonal antibody was effective after the first administration but decreased or disappeared after 8 weeks of treatment in most of the treated animals [11]. The presence of neutralizing antibodies correlated with a decreased pharmacodynamic effect (anti CD-20) and with the production of proinflammatory cytokines such as IL-1 β , IL-2, IL-6, IL-10 playing an important role in triggering the infusion reaction (Cytokine Release Syndrome, CRS) or anaphylactoid reaction clinically well documented for immunomodulatory monoclonal antibodies [11-13].

Conclusion

The preclinical safety assessment of biopharmaceuticals does not follow the same rules applied for small molecules:

- Case by case approach
- Imply a deep understanding of the mechanism of action and relevant species must be pharmacologically responsive.

The immunogenicity potential of biopharmaceuticals must be investigated at an early stage of drug development. *In vitro* and *in silico* tests are currently available and together with the preclinical toxicity assessment in the appropriate animal model, the potential for the production of ADAs including nAbs, the onset of a Cytokine Release Syndrome/Anaphylactoid reaction can be anticipated before the clinical trials thereby increasing the subject safety by implementing the safest and most conservative clinical protocols [14,15].

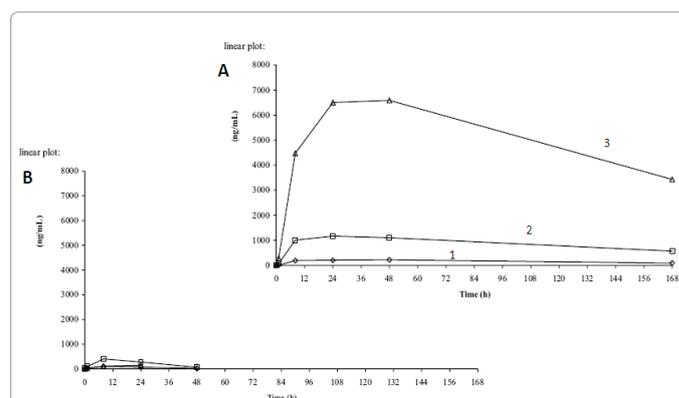


Figure 2: Influence of the production of ADAs on the systemic exposure to PEG-IFN α -2B.

Panel A: Systemic exposure to PEG-IFN α -2B in non human primates after the first subcutaneous administration.

Panel B: Systemic exposure to PEG-IFN α -2B in non human primates after the third subcutaneous administration (week 3 of treatment). 1, 2, 3: low dose, mid dose, high dose.

Binding Antibodies	Total	LD	MD	HD
Day 15 (week 2)	67% (+)	25% (+)	87% (+)	92% (+)
Day 29 (week 5)	100% (+)	100% (+)	100% (+)	100% (+)
Day 43 (week 7)	100% (+)	100% (+)	100% (+)	100% (+)
Day 85 (week 13)	100% (+)	100% (+)	100% (+)	100% (+)
Neutralizing Antibodies				
Day 15 (week 2)	52% (+)			
Day 29 (week 5)	96% (+)			
Day 43 (week 7)	89% (+)			

Table 1: Determination of binding (ADAs) and neutralizing antibodies in non human primates treated with PEG-IFN α -2B during 13 weeks by the subcutaneous route. LD: Low Dose; MD: Mid Dose; HD: High Dose.

Animals treated with Rituximab Immunogenicity results	
Binding Antibodies	
Week 4	70% (+)
Week 8	70% (+) (same animals as in week 4)
Week 8/10 recovery	50% (+) (one male and one female already positive in weeks 4 and 8)
Neutralizing Antibodies	
Week 8	60% (+) (one animal not positive but positive for binding antibodies)
Week 10 recovery	50% (+) (same animals positive for binding antibodies)

Table 2: Determination of binding (ADAs) and neutralizing antibodies in non human primates treated with Rituximab during 8 weeks by the intravenous route.

Finally, the toxicity profile and especially the NO(A)EL established after preclinical toxicity testing must be carefully interpreted in terms of establishing the MRSD. The use of the MABEL approach as recommended by the EMA could be a more appropriate approach especially in the light of the TeGenero case that changed the entire regulatory framework of the preclinical and clinical development of biopharmaceuticals [13,16-19].

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